JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 1715−1719 **1715**

Modulations of the Bcl-2/Bax Family Were Involved in the Chemopreventive Effects of Licorice Root (*Glycyrrhiza uralensis Fisch*) in MCF-7 Human Breast Cancer Cell

EUN-HYE JO,[†] HEE-DO HONG,[‡] NAM-CHIK AHN,[†] JI-WON JUNG,[†] SE-RAN YANG,[†] JOON-SUK PARK,[†] SUNG-HOON KIM,[§] YONG-SOON LEE,[†] AND KYUNG-SUN KANG*,[†]

Laboratory of Stem Cell and Tumor Biology, Department of Veterinary Public Health, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea, Korea Food Research Institute, Sungnam City, Korea, and Graduate School of East−West Medical Science, Kyunghee University, Yongin, Korea

Recently, cancer chemoprevention with strategies using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control. Genistein in soy, American ginseng, and resveratrol are well-known to have antiproliferative properties in human breast cancer. Licorice root is a botanical, a shrub native to southern Europe and Asia, which primarily has desirable qualities in sweetening and herbal medicine. In this study, licorice (*Glycyrrhiza uralensis Fisch*) root also inhibits cell proliferation in human breast cancer cell. The cell proliferation study demonstrated that licorice root reduced the proliferation of MCF-7 cells in a dose- and time-dependent manner. The extracts were fractionated in $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30), and these extracts of licorice root (50 μg/mL) induced DNA fragmentation demonstrated by Hoechst 33258 staining. Apoptosis also determined the sub-G1 accumulation by flow cytometry analysis. These results were consistent with specific cleavage of PARP and antiapoptotic protein Bcl-2 and up-regulation of proapoptotic protein Bax demonstrated by Western blotting. Our findings suggest that licorice root may have chemopreventive effects against human breast cancer through the modulation of the expression of the Bcl-2/Bax family of apoptotic regulatory factors.

KEYWORDS: Licorice; MCF-7 cell; Bcl-2; Bax; PARP; apoptosis

INTRODUCTION

Breast cancer is the most common cancer (excluding nonmalignant skin melanomas), the second leading cause of cancer deaths among women, and the leading cause of death in women ages 40−55 years; it is second only to lung cancer for overall cancer-related deaths (*1*). However, recently in the U.S.A., a large trial that demonstrated a reduction of approximately 50% in the risk of developing breast cancer led to Food and Drug Administration approval of tamoxifen as a preventive agent in women at increased risk (*2*). The chemoprevention with tamoxifen or raloxifene and extracts of herbs or plants has received more attention for the treatment of breast cancer mortality (*3*−*5*). We can find many of these chemicals in oriental herbs or plants. The licorice root has long been employed in Western countries as a flavoring and sweetening agent, as well as a demulcent and expectorant. In oriental medicine, licorice root has been known to possess various pharmaceutical functions, including detoxification, antiulcer, antiinflammation, antiviral, antiatherogenic, and anticarcinogenic activities (*6*). In addition, some components of licorice root demonstrated significant antimicrobial activity in vitro (*7*, *8*) and antioxidant activity (*9*, *10*). Chinese licorice is called kanzo, and northeastern Chinese licorice and ural liquorice dwell in Northern China, Mongolia, and Siberia. A perennial glandular herb, Chinese licorice has an erect stem with short whitish hairs and echinate glandular hairs and leaves alternate fruit in a flat oblong shape, and the root used in medicine is cylindrical, fibrous, flexible, furrowed, and light yellow inside. Previous studies have demonstrated that licorice root extract has multipotent biological effects. A water extract of licorice root was found to inhibit angiogenesis (*11*), and Licochalcone-A from licorice root has antitumor activity in vitro and in vivo in a mouse skin papilloma model (*12*). In another study, licorice root (*Glycyrrhiza glabra*) has a biological activity capable of Bcl-2 phosphorylation and G2/M cell cycle arrest in human breast cancer cell line, MCF-7 (*13*), and induced apoptosis in MCF-7 and HL-60 cell lines, as demonstrated by cleavage of

* To whom correspondence should be addressed. Tel: +82-2-880-1246. Fax: +82-2-876-7610. E-mail: kangpub@snu.ac.kr.
[†] Seoul National University.
[‡] Korea Food Research Institute.
[§] Kyunghee University.

10.1021/jf035012t CCC: $27.50
Published on Web 03/02/2004

PARP (*14*). This study is the first time to reveal that the root of licorice, *Glycyrrhiza uralensis Fisch*, has chemopreventive effects in the breast cancer cell line.

In recent letters by Wang (*15*) and Marian (*16*), the consumption of whole fruits may provide the antioxidant balance needed to quench reactive oxygen species, which have been implicated in tumorigenesis. An EPIC-Norfolk prospective study showed an inverse relation between plasma vitamin C and mortality due to cancer (*17*). The diet of unbalanced single target agents may be less advantageous than a diet of whole extracts, and then, it can permit reverse reaction as cell survival of the cancer cell. Therefore, we designed a study to investigate the antitumor effects of $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30) extracts of licorice root and elucidated the potential mechanisms using an in vitro system. We found that the extracts of licorice root were able to induce apoptosis in MCF-7 cells in a dose- and time-dependent manner, which were possibly mediated through cleavage of PARP, up-regulation of Bax, and cleavage of Bcl-2.

MATERIALS AND METHODS

Materials. Licorice roots from Kyungsan Province, Korea, were purchased in the Kyungdong oriental medicine market, Seoul, Korea. The fresh roots of licorice (*Glycyrrhiza uralensis Fisch*) were washed, disintegrated, and extracted with $CHCl_3$, EtOAc, C_6H_{14}, CH_3OH-H_2O (70:30), CH_3CH_2OH, C_4H_9OH, and H_2O for 24 h. The crude extracts obtained by subjecting into silica gel chromatography were then evaporated to dryness with a rotary evaporator. We selected $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30) extracts of licorice root reducing MCF-7 cells used in this study.

Cell Culture and Treatment with Licorice Root. MCF-7 cells (ATCC, U.S.A.) were cultured in D-media (EMEM containing a 50% increase of all essential amino acids except glutamine, a 50% increase of all vitamins, and a 100% increase of all nonessential amino acids) supplemented with 10% FBS (fetal bovine serum; Gibco Laboratories, U.S.A.) and 3 mL/L PSN (penicillin/streptomycin/neomycin) antibiotic mixture (Gibco). Exponentially growing cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The stock solutions of licorice root (100 mg/mL) were dissolved in CH_3CH_2OH, and the experimental concentrations were prepared in the basal medium with a final CH_3CH_2OH concentration of 1%.

Cell Proliferation Assay. The protocol for the cell proliferation assay was described previously (*18*). All compounds were then diluted with phenol red-free D-media supplemented with 5% dextran-coated charcoal-stripped FBS (DCC-FBS; Hyclone, U.S.A.) and 3 mL/L PSN (Gibco). The cells (5×10^4 cells/mL) were plated in a six well culture plate (2 mL/well) in triplicate and allowed to attach for 24 h. The phenol red-free D-media was replaced with D-media with 5% DCC-FBS followed by incubation for 24 h, and then, the medium was removed and replaced by test medium containing the appropriate concentrations of compounds. The cell was incubated for 3 days, and the test media were changed once. The cells were then washed three times with phosphate-buffered saline and lysed with 1 mL of 0.1 N NaOH. The lysates were transferred into a 1.5 mL microcentrifuge tube and centrifuged for 2 min. The DNA content was determined by the method described previously (*19*). The OD_{260nm} value of the clear lysate was measured with a spectrophotometer (DU 650, Beckman, Fullerton, U.S.A.). All of the measurements were performed in duplicate. Results are expressed as the percentages of proliferation with respect to vehicle-treated cells.

Apoptosis Assay. The apoptotic effect of extracts of licorice root on MCF-7 cells was analyzed by nuclear DNA staining and DNA fragmentation assay. For nuclear DNA staining, control and compound-treated cells were fixed in 4% paraformaldehyde in PBS for 20 min, washed with PBS, stained with Hoechst 33258 at 1 μg/mL in PBS for 15 min. Stained cells were washed twice with PBS. The changes in nuclei were observed with a fluorescent microscope (Olympus, U.S.A.) through a UV filter.

Flow Cytometric Analysis of Apoptosis. MCF-7 cells in the exponential phase of growth were treated with all of the extracts of licorice root (50 μg/mL) for the indicated times, then harvested by trypsinization, and washed twice with ice-cold PBS and fixed by 70% ethanol at −20 °C for at least 30 min. The fixed cells were then washed twice with ice-cold PBS and stained with 50 μg/mL of propidium iodide in the presence of 50 μg/ml RNase A for 30 min. Cell cycle distribution was analyzed using FACS Calibur (Becton & Dickinson, U.S.A.). Data from 10 000 cells per sample were collected and analyzed using the Cell Fit Cell analysis program.

Western Blot Analysis. MCF-7 cells were grown in a 10 cm dish, and when the cell density reached 80−90% confluence, cells were treated with extracts of licorice root (50 μg/mL) for the indicated times. The cells were then washed once with ice-cold PBS and lysed with lysis buffer (20% SDS containing 2 mM phenymethylsulgonyl fluoride, 10 mM iodoacetoamide, 1 mM leupeptin, 1 mM antipain, 0.1 mM sodium orthovanadate, and 5 mM sodium fluoride) for 10−20 min. The lysates were sonicated three times at 10 s intervals, aliquoted, and stored at −20 °C. The protein concentration was determined by the Bio-Rad DC protein assay (Bio-Rad Laboratories, U.S.A.). Equal amounts of protein (20 μg/lane) were subjected to 12% SDS−PAGE and transferred to nitrocellulose membranes. The membranes were subsequently incubated with the corresponding primary antibodies, as indicated: a mouse anti-PARP monoclonal antibody (Santa Cruz Biotechnology, U.S.A.), a mouse anti-Bcl-2 monoclonal antibody (Zymed Laboratories Inc., CA); and a rabbit anti-Bax antibody (Sigma). Antibody recognition was detected with the respective secondary antibody, ether anti-mouse IgG, or anti-rabbit IgG antibodies linked to horseradish peroxidase (Zymed Laboratories Inc.). Antibody-bound proteins were detected by the ECL western blotting analysis system (Amersham Pharmacia Biotech U.K. Limited).

RESULTS

Extracts of Licorice Root Decreased Cell Proliferation in MCF-7. To analyze the inhibition of trichloromethane, ethyl acetate, 70% methanol, and hexane extracts of licorice root on the growth of MCF-7 cells, we measured DNA synthesis in the presence of licorice root. As shown in **Figure 1**, trichloromethane and ethyl acetate extracts of licorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner. After 72 h of treatment, the 50 μg/mL of trichloromethane extract nearly caused a 63% inhibition and the 50 μg/mL of ethyl acetate extract nearly appeared an 83% inhibition of cell growth as compared with control. Also, 70% methanol and hexane extracts of licorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner in **Figure 1**. After 72 h of treatment, at the same dose, this 70% methanol extract caused nearly a 62% inhibition and the hexane extract appeared nearly an 80% inhibition of cell growth as compared with control. These results were used in all further experiments.

Extracts of Licorice Root Induced Apoptosis in MCF-7 Cells. To analyze the contribution of apoptosis to this process, Hoechst 33258 staining was performed. When MCF-7 cells were treated with 50 μg/mL licorice root extracts for 48 h, cells exhibited typical morphological changes of apoptosis. As shown in **Figure 2**, the test compound induced chromatin condensation and nuclear fragmentation (arrows). The cells shrank, turned around, and had a relatively smaller volume than control cells.

Considering that licorice root decreased cell proliferation and induced cell death, cell cycle analyses were performed with flow cytometry. As shown in **Figure 3**, cells accumulated in the sub-G1 phase gradually from 24 to 72 h after treatment with the test compound, whereas the number of cells in G1 phase decreased in the same manner. All of the licorice root extracts were found to be effective on the apoptosis of MCF-7 cells.

Effects on PARP Expression and Cleavage in MCF-7 Cells. To assess the role of PARP in this apoptotic process, the expression of PARP was examined by western blot analysis. As shown in **Figure 4**, the 116 K_d PARP was cleaved to its

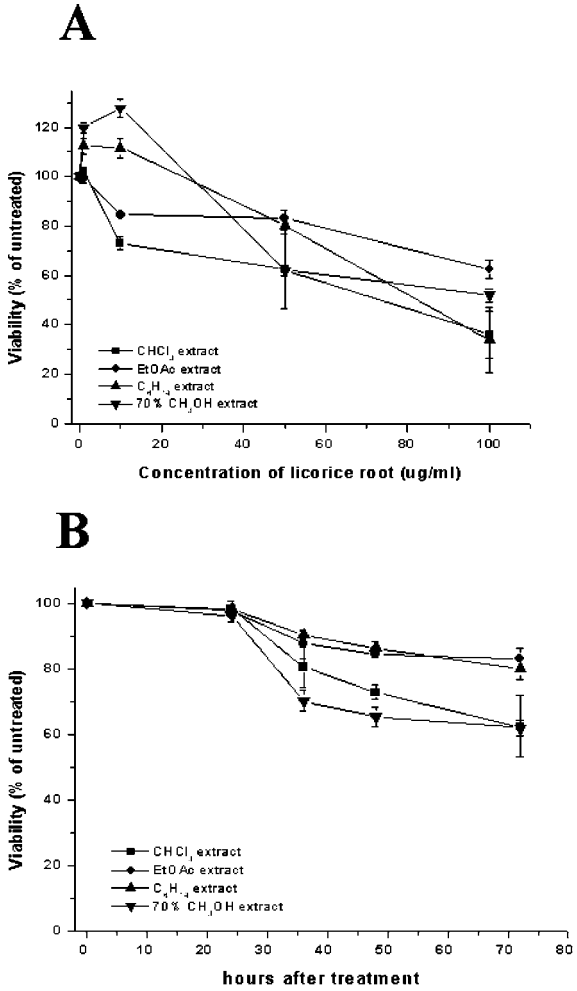

Figure 1. Inhibition of proliferation by licorice root. Cells were treated with various concentrations of test compound for 72 h (**A**) and various times at 50 μg/mL licorice root extracts (**B**). Cell viability was determined by cell proliferation assay. Results are expressed as percentages of proliferation as compared with untreated control (mean ± SD, $n = 2$).

active 85 K_d in MCF-7 cells treated with trichloromethane, ethyl acetate, and hexane and 70% methanol extracts of licorice root induced PARP degradation fragments in MCF7 cells. This result showed that licorice root induced apoptosis, since treatment with licorice root resulted in cleavage of PARP, the substrate of several ICE-like proteases.

Effects on Bcl-2/Bax Expression. To determine the effect of licorice root on apoptotic pathways, we evaluated transcription factor Bcl-2 and Bax expression by western blot. As demonstrated in **Figure 4**, licorice root caused Bcl-2 cleavage in MCF-7 cells and the Bax protein level was increased 72 h after treatment. The 26 K_d Bcl-2 was cleaved to its active 22 K_d in MCF-7 cells treated with licorice root. The results suggested that trichloromethane, ethyl acetate, hexane, and 70% methanol extracts of licorice root induced apoptosis in MCF-7 cells and might be mediated through the cleavage of Bcl-2 and up-regulation of the Bax pathway.

DISCUSSION

Apoptosis or programmed cell death is an essential physiological process that plays a critical role in development and tissue homeostasis (*20*). The goal of cancer chemoprevention is to inhibit the induction and suppress the progression of preneoplastic lesions to invasive cancer by using specific natural

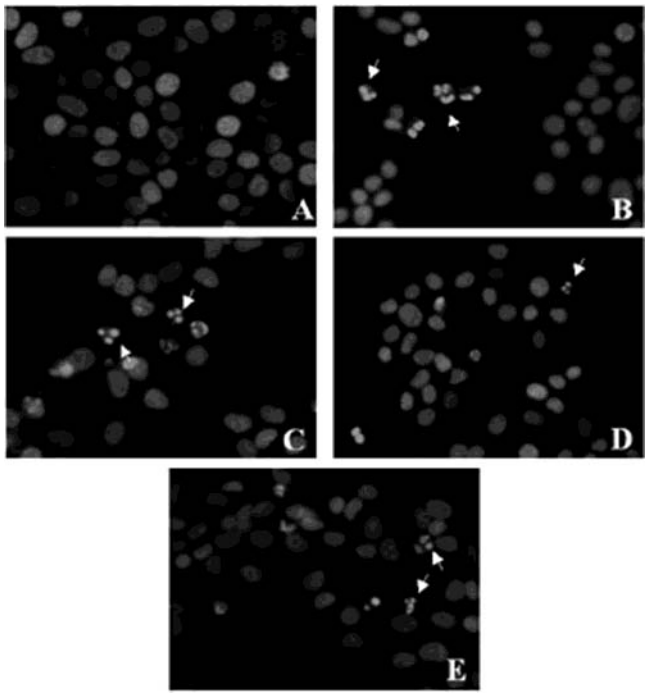

Figure 2. Detection of apoptotic morphological changes in MCF-7 cells treated with 50 μg/mL all extracts of licorice root. Nuclei were stained with Hoechst 33258 and examined by fluorescence microscopy. Normal MCF-7 cells (**A**) and treated with $CHCl_3$ (**B**), EtOAc (**C**), C_6H_{14} (**D**), and CH_3OH–H_2O (70:30) (**E**) extracts of licorice root for 48 h.

or synthetic chemicals. Further understanding of the effects of potential chemopreventive agents on specific components of the pathways that lead to apoptosis may provide a rational approach to use such agents alone or in combination with other agents to enhance apoptosis as a strategy for effective chemoprevention of cancer (*21*). There are two main pathways leading to apoptosis. The first of these depends on the participation of mitochondria and the second involved in the interaction of a death receptor with its ligand. Pro- and antiapoptotic members of the Bcl-2 family regulate the mitochondrial pathway (*22*).

In this study, using MCF-7 human breast cancer cell lines, we have shown the effect of licorice root, *Glycyrrhiza uralensis Fisch*, on cell proliferation and on the induction of apoptosis in a cell specific manner. In these results, we have found that $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30) extracts of licorice root decreased cell viability in MCF-7 in concentration- and time-dependent manners. We demonstrated that $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30) extracts of licorice root induced apoptosis through the cleavage of Bcl-2 protein and Bax protein up-regulation. Cellular stress induced proapoptotic Bcl-2 family members to translocate from the cytosol to the mitochondria, where they induced the release of cytochrome c, while the antiapoptotic Bcl-2 proteins worked to prevent cytochrome c release from mitochondria, and thereby preserved cell survival (*21*). Especially, unlike Bcl-2, the cleaved Bcl-2 fragment was no longer functional for dimerization with either Bcl-2 or Bax (*23*).

A recent study showed that alcohol extracts of licorice root (*Glycyrrhiza glabra*) could inhibit cell growth and induce cell cycle arrest at the transition G2/M phase in tumor cell lines (*22*). However, we could not observe that the extracts of licorice root were capable of inducing cell cycle arrest in MCF-7 cells. To confirm this result, flow cytometric analysis was performed. All extracts of licorice root did not induce cell cycle arrest in any phase. However, in the sub-G1 population, the number of

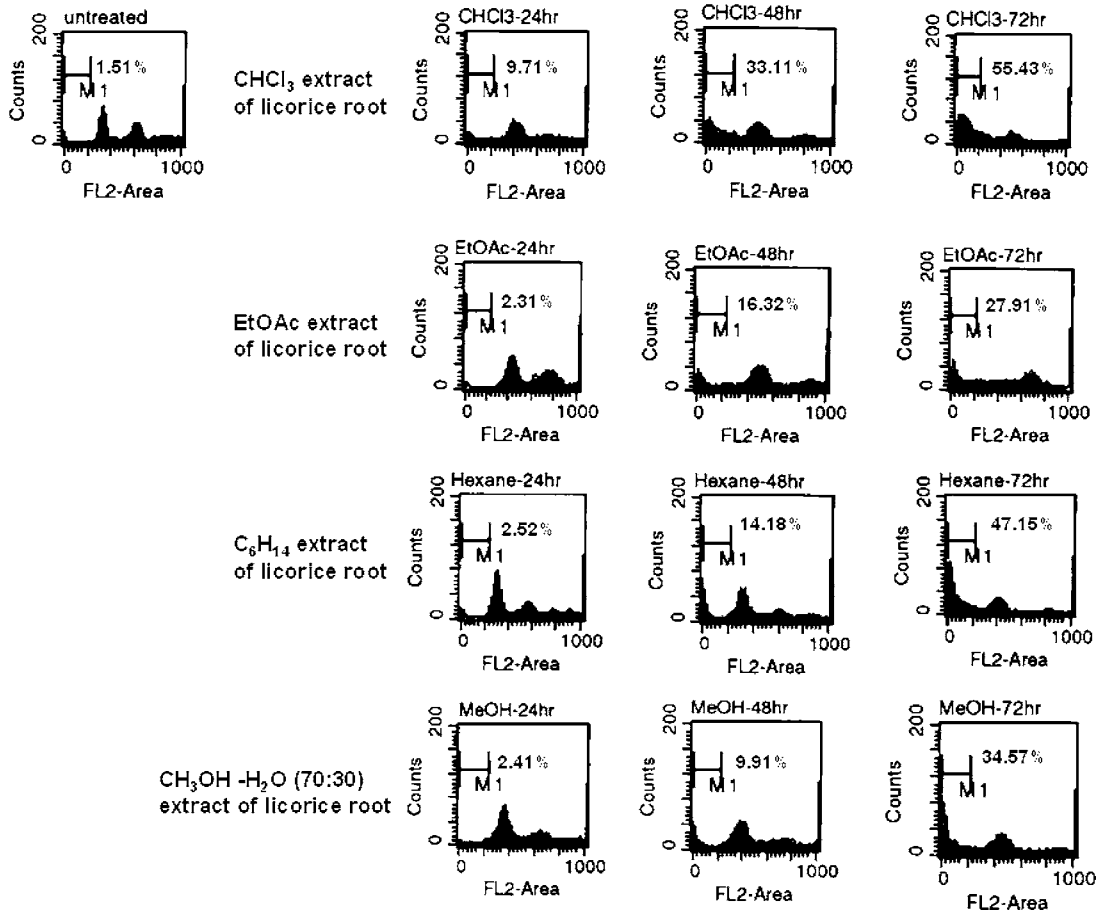

Figure 3. Detection of apoptotic cells by flow cytometry in MCF-7 cells treated with 50 μg/mL all extracts of licorice root.

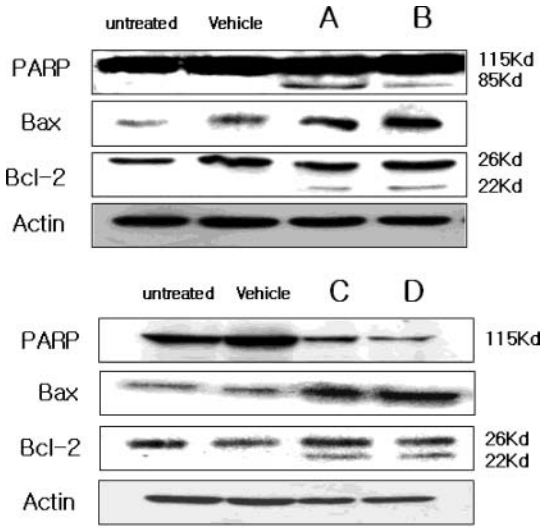

Figure 4. Effect of licorice root on expression of PARP, Bax, and Bcl-2 proteins. Cells were treated with 50 μg/mL extracts of licorice root. MCF-7 cells were treated with 50 μg/mL of $CHCl_3$ (**A**), EtOAc (**B**), C_6H_{14} (**C**), and CH_3OH–H_2O (70:30) (**D**) extract of licorice root. Total cellular proteins were prepared, and western blot was performed with an antibody specific for corresponding proteins.

apoptotic cells increased significantly in MCF-7 cells treated with licorice root. Accumulating with sub-G1 DNA content is consistent with the result of the proliferation assay because nonsub-G1 cells decreased in the way of viability treated with licorice. In addition, a nuclear enzyme involved in DNA repair and maintenance of genome integrity and posttranslational ribosylation of proteins, whereby apoptosis occurs, PARP cleaves several substrates. This occurred with cleavage of 116 K_d PARP to 85 K_d proteolytic fragments in MCF-7 cells treated with $CHCl_3$ and EtOAc extracts of licorice root. Also, C_6H_{14} and CH_3OH-H_2O (70:30) extracts of licorice root just induced PARP degradation fragments in MCF-7 cells. It appeared that the pattern of PARP cleavage differed from that observed in MCF-7 cells treated with $CHCl_3$ and EtOAc extracts of licorice root and did not lead to a persistent fragment. It could be hypothesized that PARP degradation continues after its first cleavage.

In summary, $CHCl_3$, EtOAc, C_6H_{14}, and CH_3OH-H_2O (70:30) extracts of licorice root induce apoptosis through the overexpression of Bax and the cleavage of Bcl-2 in human breast cancer cells. It is concluded that the root of licorice, *Glycyrrhiza uralensis Fisch*, might be a good chemopreventive natural product for human breast cancer.

LITERATURE CITED

(1) Parker, S. L.; Tong, T.; Bolder, S.; Wingo, P. A. Cancer Statistics. *C. A. Cancer J. Clin.* **1998**, *48*, 26−29.

(2) Decensi, A.; Costa, A. Recent advances in cancer chemoprevention, with emphasis on breast and colorectal cancer. *Eur. J. Cancer* **2000**, *36*, 694−709.

(3) Christov, K.; Ikui, A.; Shilkaitis, A.; Green, A.; Yao, R.; You, M.; Grubbs, C.; Steele, V.; Lubet, R.; Weinstein, I. B. Cell proliferation, apoptosis, and expression of cyclin D1 and cyclin E as potential biomarkers in tomoxifen-treated mammary tumors. *Breast Cancer Res. Treat.* **2003**, *77*, 253−264.

(4) Hu, H.; Ahn, N. S.; Yang, X.; Lee, Y. S.; Kang, K. S. Ganoderma lucidum extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. *Int. J. Cancer* **2002**, *102*, 250−253.

(5) Cho, J. H.; Cho, S. D.; Hu, H. B.; Kim, S. H.; Lee, Y. S.; Kang, K. S. The roles of ERK1/2 and p38 MAP Kinases in the preventive mechanisms of mushroom phellinus linteus against the inhibition of gap junctional intercellular communication by hydrogen peroxide. *Carcinogenesis* **2002**, *23*, 1163−1169.
(6) Wang, Z. Y.; Nixon, D. W. Licorice and cancer. *Nutr. Cancer* **2001**, *39*, 1−11.
(7) Demizu, S.; Kajiyama, K.; Takahashi, K.; Hiraga, Y.; Yamamoto, S.; Tamura, Y. Antioxidant and antimicrobial constituents of licorice: Isolation and structure elucidation of a new benzofuran derivative. *Chem. Pharm. Bull.* **1998**, *36*, 3474−3479.
(8) Friis-Møller, A.; Chen, M.; Fuursted, K.; Christensen, S. B.; Kharazmi, A. In Vitro Antimycobacterial and Antilegionella Activity of Licochalcone A from Chinese Licorice Roots. *Planta Med.* **2002**, *68*, 416−419.
(9) Belinky, P. A.; Aviram, M.; Fuhrman, B.; Rosenblat, M.; Vaya, J. The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. *Atherosclerosis* **1998**, *137*, 49−61.
(10) Vaya, J.; Belinky, P. A.; Aviram, M. Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radical Biol. Med.* **1997**, *23*, 302−313.
(11) Kobayashi, S.; Miyamoto, T.; Kimura, I.; Kimura, M. Inhibitory effect of isoliquiritin, a compound in licorice root, on angiogenesis in vivo and tube formation in vitro. *Biol. Pharm. Bull.* **1995**, *18*, 1382−1386.
(12) Shibata, S.; Inoue, H.; Iwata, S.; Ma, R. D.; Yu, L. J.; Ueyama, H. Inhibitory effects of licochalcone A isolated from Glycyrrhiza inflata root on inflammatory ear edema and tumour promotion in mice 57. *Planta Med.* **1991**, 221−224.
(13) Rafi, M. M.; Vastano, B. C.; Zhu, N.; Ho, C.-T.; Ghai, G.; Rosen, R. T. Novel Polyphenol Molecule Isolated from Licorice Root (Glycrrhiza glabra) Induces Apoptosis, G2/M Cell Cycle Arrest, and Bcl-2 Phosphorylation in Tumor Cell Lines. *J. Agric. Food Chem.* **2002**, *50*, 677−684.
(14) Rafi, M. M.; Rosen, R. T.; Vassil, A.; Ho, C. T.; Zhang, H.; Ghai, G. Modulation of bcl-2 and cytotoxicity by licochalcone-A, a novel estrogenic flavonoid. *Anticancer Res.* **2000**, *20*, 653−658.
(15) Wang, H.; Cao, G.; Prior, R. L. Total Antioxidant Capacity of Fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.
(16) Marian, V.; Eberhardt; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apple. *Nature* **2000**, *405*, 903−904.
(17) Khaw, K. T.; Bingham, S.; Welch, A. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. *Lancet* **2001**, *357*, 657−663.
(18) Soto, A. M.; Chung, K. L.; Sonnenschein, C. The pesticides endosulfan, toxaphene, and dieldrin have strogenic effects on human estrogen-sensitive cells. *Environ. Health Perspect.* **1994**, *102*, 380−383.
(19) Kang, K. S.; Sun, W.; Nomata, K.; Morita, I.; Cruz, A.; Liu, C. J. Involvement of tyrosine phosphorylation of p185(c-erbB2/neu) in tumorigenicity induced by X-rays and the neu oncogene in human breast epithelial cells. *Mol. Carcinog.* **1998**, *21*, 225−233.
(20) Zimmermann, K. C.; Bonzon, C.; Green, D. R. The machinery of programmed cell death. *Pharmacol. Ther.* **2001**, *92* (1), 57−70.
(21) Sun, S. Y. Apoptosis induction by chemopreventive agents. *Drug News Perspect.* **2001**, *14* (2), 75−80.
(22) Rafi, M. M.; Vastano, B. C.; Zhu, N.; Ho, C. T.; Ghai, G.; Rosen, R. T. Novel polyphenol molecule isolated from licorice root (Glycrrhiza glabra) induces apoptosis, G2/M cell cycle arrest, and Bcl-2 phosphorylation in tumor cell lines. *J. Agric. Food. Chem.* **2002**, *50*, 677−684.
(23) Lin, H.; Zhang, X. M.; Chen, C.; Chen, B. D. Apoptosis of Mo7e Leukemia cells Is associated with the cleavage of Bcl-2 into a shortened fragment that is not functional for heterodimerization with Bcl-2 and Bax. *Exp. Cell Res.* **2000**, *261*, 180−186.

Received for review September 5, 2003. Revised manuscript received January 12, 2004. Accepted January 20, 2004. This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry (200001-03-3-CG000, 203004-03-HD110), Republic of Korea. The Research Institute also supported this work for Veterinary Science, Seoul National University.

JF035012T